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Plasma D-Dimer Measurement in Patients with Suspected DVT – a Means of Avoiding Unnecessary Venography

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Objectives: *To assess the applicability of plasma D-dimer levels in the exclusion of a DVT.*
Design: *Consecutive cohort of patients.*
Materials and Methods: *Eighty consecutive patients presenting to the radiology department with a clinical diagnosis of DVT were included. Citrated blood samples were taken from all patients before radiological investigation, plasma isolated and frozen for subsequent testing. The patients then underwent venography (duplex scan was also used in some cases). Plasma samples were tested using the NycoCard D-Dimer. NycoCard Reader was used to estimate the D-dimer concentrations.*
Results: *A DVT was diagnosed in 29 cases (36.7%). Plasma D-dimer levels had a sensitivity of 96% (only one false negative), specificity of 40%, positive predictive value of 48%, and negative predictive value of 95% when compared to venography.*
Conclusions: *A normal plasma D-dimer level could be used as an exclusion test for DVT avoiding complications of venography and saving time and money.*

Key Words: Deep venous thrombosis; D-dimer; Venography.

Introduction

Clinical diagnosis of deep vein thrombosis (DVT) is unreliable with an accuracy of approximately 50%.[1,2] Approximately 50% of patients will therefore undergo investigation for DVT unnecessarily with either venography or Doppler ultrasound. Venography, regarded as the "gold standard", has the advantage of being invasive, costly, time-consuming and demanding technical expertise.[3] Furthermore, it is not without side-effects,[4] is less reliable in cases of recurrent DVT,[5] and may fail to visualise some segments of the venous system in 10–30% of cases.[6] Doppler ultrasound (with an accuracy of 80–90% compared to venography) is very operator-dependent.[6] For a blood test to be useful in a clinical setting for screening patients presenting with a suspected DVT it would have to be rapid, sensitive, and easy to perform.

Once a thrombus has formed in the deep veins of the leg it is broken down by the fibrinolytic system. D-dimers are unique fragments derived from fibrin by the hydrolytic action of plasmin.[7] Thus the presence of a thrombus may be associated with an increase in the plasma level of D-dimer.[8] D-dimer levels are, however, increased in a wide variety of conditions,[9] and therefore cannot be used to predict the presence of a DVT. Normal levels of D-dimer may, however, be used to "rule out" DVT.[8] Various studies have confirmed that a low concentration of D-dimer measured by the ELISA technique (usually less than 500 µg/l) might be used to exclude clinically suspected venous thromboembolism.[10] Most of these assays were, however, carried out in specialist research laboratories.

The aim of this study was to determine the usefulness of a rapid plasma immunofiltration assay (NycoCard D-Dimer) in excluding the diagnosis of a DVT in a clinical setting.

Method

Local ethical committee approval was obtained for the study. Eighty consecutive patients presenting to the radiology department with a presumptive diagnosis

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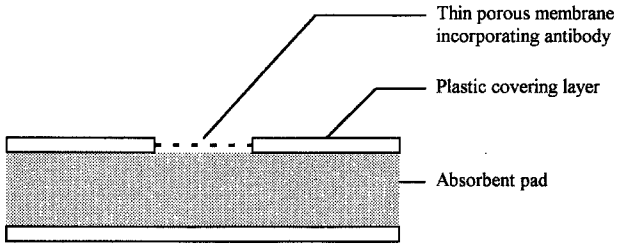

Fig. 1. Diagram showing the structure of the NycoCard.

of lower limb DVT were entered into the study after informed consent. All the patients were ambulatory, had not undergone surgery or major trauma and were without significant underlying disease (e.g. cancer, rheumatoid arthritis etc.). The duration of symptoms was noted prior to presentation to the hospital, but once in hospital the radiological examination was carried out within 24 h. A 5 ml blood sample was collected into a sterile tube, containing sodium nitrate as anticoagulant, before undergoing radiological tests. All patients were investigated with either venography or duplex scanning (combined with venography in uncertain cases – especially in cases of lower leg DVT). The extent of the DVT (calf or proximal) was noted. All investigations were carried out by consultant radiologists, experienced in venography and duplex scanning, blinded to the results of the D-dimer assay.

Plasma was prepared from the citrated blood by centrifugation at 2500 g for 15 minutes and tested either on the same day or frozen and tested in batches.

NycoCard D-Dimer (Nycomed (UK) Ltd) utilises a gold antibody conjugate in an immunofiltration test principle which produces a reddish purple colour at pathological D-dimer concentrations. The level of plasma D-dimer can be estimated in the range of 0.5–8 mg/l. The main components of the card are shown in Figure 1. The thin porous membrane carries the monoclonal antibodies reacting with D-dimer-configured molecules. The assay is performed as follows: the membrane is activated by the addition of 50 µl of a washing solution, followed by 50 µl of citrated plasma sample. After the liquid has been taken up by the absorbent pad 50 µl of a conjugate between the same D-dimer-reactive antibody and 4 nm gold colloids is added, followed by 50 µl of washing solution. The colour intensity was used to estimate the concentrations of D-dimer. This was done with both a NycoCard Reader and visually using a reference colour chart with five zones of colour corresponding to D-dimer concentrations of 0.5, 1.0, 2.0, 4.0, and 8.0 mg/l. These measurements were performed by two independent observers blinded to each other's results (one using the NycoCard Reader and the other using the visual reference chart). The entire test procedure is completed in less than 2 min. A NycoCard D-dimer concentration of <0.5 mg/l was used to indicate normal levels (i.e. a negative test).

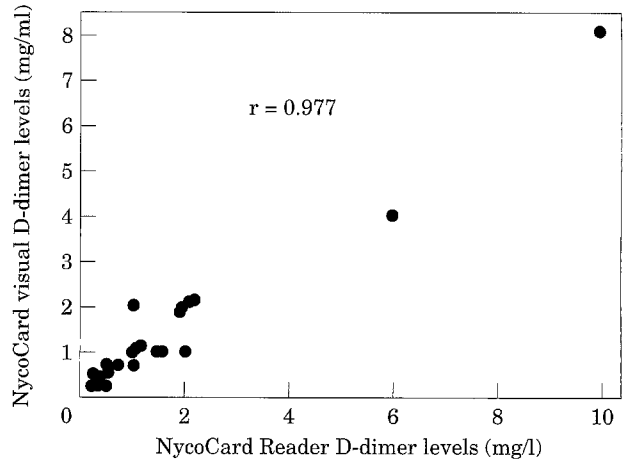

Fig. 2. Correlation between D-dimer levels as determined by visual reading of the NycoCard and by using the NycoCard Reader.

Results

Of the 80 specimens one was rejected because it was grossly lipaemic and would not filter through the porous membrane. Of the remaining 79 cases there were 31 men and 48 women with a median age (range) of 63 (32–89) years. Duration of symptoms prior to investigation was recorded in 32 cases and was a median (range) of 6.5 (2–90) days. A DVT was diagnosed (using venography or duplex) in 29 (36.7%) of cases, 16 involving the calf veins only and 13 involving the iliofemoral segment as well. Venography alone was used in 71 cases, duplex scanning alone in six cases and both modalities in two cases. The two groups (DVT positive and negative) were well matched for sex ratio (Chi-squared test $\chi^2=0.088$; $p>0.5$) and age (Mann-Whitney test $p=0.62$). However, some patients had already been started on treatment with intravenous heparin. There were 14 (48.3%) in the DVT positive group and 13 (26%) in the DVT negative group ($\chi^2=4.05$; $0.02<p<0.05$).

Figure 2 shows the correlation between the D-dimer levels as determined by visual scoring against the NycoCard Reader. Although an excellent correlation is seen ($r=0.977$) on three occasions, visual reading was negative, whereas the NycoCard Reader gave a positive result.

There was no correlation between duration of symptoms and D-dimer levels, nor between duration of symptoms and the presence or absence of a DVT.

Table 1. Data showing DVT positive and negative cases against D-dimer test results.

	DVT positive	DVT negative	Total
D-dimer positive*	28	30	58
D-dimer negative**	1	20	21
Total	29	50	79

*D-dimer test was considered positive at levels ≥ 0.5 mg/l.
**D-dimer test was considered negative at levels <0.5 mg/l.

Table 1 shows the D-dimer levels against the radiological investigation results. Using this table the sensitivity was 96%, the specificity 40%, the positive predictive value 48%, and the negative predictive value 95%.

Discussion

The usefulness of plasma D-dimer levels as an exclusion test for DVT has been recognised for some time and has been studied using commercially available ELISA or latex agglutination tests.[10] Enzyme immunoassays are precise, quantitative, analytical tools, but are time-consuming and require technical skill. The latex tests are simple and rapid, but are semi-quantitative and not easily read.[11] The NycoCard employs a filtration-type immunoassay that combines quantitative properties with rapidity and simple interpretation of results. It has been validated against an ELISA.[11,12]

Bounameaux *et al.*[10] have reviewed the results of ELISA against all diagnostic methods for DVT and report on average sensitivity of 96.8%, specificity of 35.2%, positive predictive value of 44.3%, and negative predictive value of 95.4%. The results obtained in our study are comparable to these. The NycoCard has been tested in a hospital setting by Dale *et al.*[12] They report results similar to those in this study but with a lower specificity of 23% and negative predictive value of 87%.

The effect of heparin needs to be considered. There were significantly more patients in the DVT positive group on heparin. Heparin has been reported to cause a decrease in the recovery of D-dimer using the NycoCard by Gogstad *et al.*[11] and to have no effect on D-dimer levels as measured by ELISA.[13] The one patient who was negative on testing but in fact had no iliofemoral DVT was on heparin. If heparin causes a decrease in the measured value of D-dimer, the level recorded would have been higher had the patient not been on heparin and the test would have been positive. One could, therefore, suggest that all patients should be tested before anticoagulation is started. This is clearly feasible as the test takes less than 2 min to perform. Overall, however, taking into account time to deliver the specimen, centrifugation and testing the turnaround time is approximately 1 h.

Of our sample of 79 patients, 20 underwent venography, with its attendant complications, which could have been avoided had a D-dimer estimation been made. Furthermore, a saving could have been made in terms of money and of the radiologists' time. A venogram takes 20–30 min and costs approximately £65 per leg. Thus, of the 79 patients, 20 (25% of the case load) could have avoided venography – representing a saving of 10 h and £1300. These patients would also avoid exposure to radiation and the potential complications of venography. This compares to a cost of approximately £10 per D-dimer analysis in the department of haematology. The kits cost £125 for 48 tests (£2.60 per test).

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References

1 Cranley JJ, Canos AJ, Sull WJ. The diagnosis of deep vein thrombosis. *Arch Surg* 1976; **111**: 34–36.
2 O'Donnell TF, Abbott WM, Athanasoulis CA, Millan VG, Callow AD. Diagnosis of deep vein thrombosis in the outpatient by venography. *Surg Gyn Obs* 1980; **150**: 69–74.
3 Hansson PO, Eriksson H, Eriksson E, Jagenburgh R, Lukes P, Risberg B. Can laboratory testing improve screening strategies for deep vein thrombosis at an emergency unit? *J Intern Med* 1994; **235**: 143–151.
4 Lensing AWA, Prandoni P, Buller HR, Casara D, Cogo A, ten Cate JW. Lower extremity venography with Iohexol: Results and complications. *Radiology* 1990; **177**: 503–505.
5 Hull RD, Carter CJ, Jay RM, Ockelford PA, Hirsch J, Turpie AG *et al.* The diagnosis of acute, recurrent, deep vein thrombosis: A diagnostic challenge. *Circulation* 1983; **67**: 901–906.
6 Wheeler HB, Anderson FA. Diagnostic approaches for deep vein thrombosis. *Chest* 1986; **89**: 407s–412s.
7 Gaffney PJ. D-dimer. History of the discovery, characterization and utility of this and other fibrin fragments. *Fibrinolysis* 1993; **7** Suppl 2: 2–8.
8 Devine DV. Utility of D-dimer measurement in deep venous thrombosis. *Fibrinolysis* 1993; **7** Suppl 2: 12–16.
9 Rowbotham B, Whitaker AN, Masci P. D-dimer antibodies. Powerful reagents for the study of human thrombosis and fibrinolysis. *Fibrinolysis* 1993; **7** Suppl 2: 9–11.
10 Bounameaux H, de Moerloose P, Perrier A, Reber G. Plasma measurement of D-Dimer as diagnostic aid in suspected venous thromboembolism: An overview. *Thrombosis & Haemostasis* 1994; **71**: 1–6.

11 Gogstad GO, Dale S, Brosstad F, Brandness O, Holtlund J, Mork E *et al.* Assay of D-dimer based on immunofiltration and staining with gold colloids. *Clin Chem* 1993; **39**: 2070–2076.
12 Dale S, Gogstad GO, Brosstad F, Godal HC, Holtlund K, Mork E *et al.* Comparison of three D-dimer assays for the diagnosis of DVT: ELISA, latex and an immunofiltration assay (NycoCard D-dimer). *Thrombosis and Haemostasis* 1994; **71**: 270–274.
13 Elias A, Bonfils S, Daoud-Elias M, Gautheir B, Sie P, Boccalon H *et al.* Influence of long term oral anticoagulants upon prothrombin fragments 1 + 2, thrombin-antithrombin III complex and D-Dimer levels in patients affected by proximal deep vein thrombosis. *Thrombosis and Haemostasis* 1993; **69**: 302–305.

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